TITLE: Purification and properties of L-asparaginase produced by Aspergillus niger, S-48 TAT, the causal fungus of biodeterioration inside Tut Ankhamen Tomb (TAT)

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AB The purification and properties of L-asparaginase (I) produced by A. niger S-48 TAT, the causal factor of biodeterioration inside the Pharaoh Tutankhamen tomb (TAT), is reported. The purification procedure involved cell-free filtrate preparation (specific activity of 8.92 U/mg protein/mL), fractional precipitation with (NH4)2SO4, (specific activity of 21.05 U/mg protein/mL corresponding to a 2.35-fold purification), dialysis against distilled water followed by dialysis against sucrose crystals, (specific activity of 36.92 U/mg protein/mL, corresponding to a 5.7-fold purification) and finally applying a column of Sephadex G-100 (specific activity of 61.0 U/mg protein/mL corresponding to a 6.83-fold purification). The regulatory role of different buffers applied at different pH values revealed that purified I exhibited a maximum specific activity of 62.8 U/mg protein/mL in the presence of citrate-phosphate buffer pH 6.6, followed by citrate buffer pH 6.0 (specific activity of 55.46 U/mg protein/mL) and then Tris-HCl buffer pH 7.4 which revealed an obvious decrease in the specific activity (34.16 U/mg protein/mL). By testing purified I

in the presence of different substrates, it was found that the highest activity was obtained by using the most preferable one, i.e., L-asparagine, followed by L-aspartic acid, L-glutamine, and L-glutamic acid, whereas L-arginine, L-ornithine, L-threonine and L-citrulline showed negligible or inhibitory effects toward the purified enzyme activity. Moreover, the application of different heavy metal cations (in the form of chloride salts in addition to KCN) as activators and/or inhibitors indicated that $CaCl_2$, $NH_4Cl$, $BaCl_2$, and $MnCl_2$ promoted I activity, whereas $AlCl_3$, KCN, $NiCl_2$, $ZnCl_2$, $FeCl_2$, and $MgCl_2$ had deleterious effects on enzyme activity. Purified I was tested at different incubation temps., and showed obvious activity within the temperature range of 22.5-45° with a maximum at 30°.